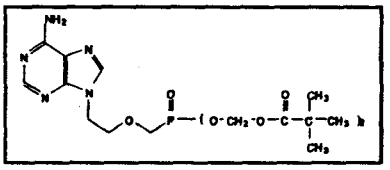
CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-449

PHARMACOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY COVER SHEET



NDA number: 21-449

Review number: 000

Sequence number/date/type of submission: 000/March 25, 2002/original application

Information to sponsor: No

Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Manufacturer for drug substance:

[redacted]

Reviewer name: Pritam S. Verma, Ph.D.

Division name: DAVDP
HFD-530

Review completion date: April 2, 2002

Drug:

Trade name: [redacted] (proposed)
Generic name: Adefovir dipivoxil (bis-POM PMEA)
Code name: GS-0840
Chemical Names: 9-[2-(bispivaloyloxymethyl)-phosphonylmethoxyethyl]-adenine
CAS registry number: 142340-99-6
Molecular formula/molecular weight: $C_{20}H_{32}N_5O_8P$/501.48

Relevant INDs/NDAs/DMFs: [redacted]

Drug class: Nucleotide analog

Indication: Treatment of chronic hepatitis B (HBV) infection

Clinical formulation: 10 mg adefovir dipivoxil tablet contains the following inactive ingredients: pre-gelatinized starch [redacted] croscarmellose sodium [redacted] lactose monohydrate [redacted] talc [redacted] and magnesium stearate [redacted]

Route of administration: Oral

Proposed use: Treatment of HBV infection

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

APPEARS THIS WAY
ON ORIGINAL

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability: Adequate information

B. Recommendation for Nonclinical Studies: Adequate

C. Recommendation on Labeling: labeling issues will be dealt with separately

II. Summary of Nonclinical Findings

Single dose toxicology studies of adefovir dipivoxil and adefovir: the single dose studies were conducted in mice, rats, rabbits and monkeys. Adefovir dipivoxil was administered via oral gavage to rats at single dose levels of 0, 24, 75 or 225 mg/kg. A single oral gavage dose of 75 mg/kg bis-POMPMEA in rats may be considered a NOEL. Based on an equivalent body surface area factor, an equivalent dose in humans would be approximately 12.17 mg/kg. The acute lethal dose in rats exceeded 225 mg/kg. CD-1 mice were administered single iv doses of adefovir at 0, 250 or 500 mg/kg. The estimated minimum lethal dose in the mouse was > 500 mg/kg. Sprague-Dawley rats received adefovir at single iv dose levels of 0, 100, 250, 500 or 1000 mg/kg. The estimated single minimum lethal dose in the rat was 500 mg/kg. New Zealand White rabbits were administered sc or im injections of adefovir at dose levels of 0, 25, 50 or 75 mg/kg. The compound was found to be a local irritant in the rabbit. No mortalities occurred in the study. Cynomolgus monkeys were administered single iv doses of adefovir at dose levels of 0, 150 or 500 mg/kg. Mortalities occurred in the monkey at 150 mg/kg dose level and higher.

Multiple-dose toxicology studies-adefovir dipivoxil: the subchronic oral toxicity of adefovir dipivoxil was evaluated in Swiss-Webster mice at dose levels of 0, 5, 20 or 80 mg/kg/day for 4-weeks. A NOAEL in this study could not be identified. The NOAEL should be somewhere between 0 and 5 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose in humans would be between 0.0 and 0.40 mg/kg/day. A 13-week oral toxicology study in CD-1 mice was conducted at dose levels of 0, 10, 30 or 100 mg/kg/day. Based on the histopathological changes present in the liver (hepatocellular karyo/cytomegaly, single cell necrosis) and spleen (lymphoid necrosis/atrophy) in the low dose group animals, a NOAEL in this study could not be identified. The NOAEL should be somewhere between 0 and 10 mg/kg/day dose levels. Based on a body surface area conversion factor, an equivalent dose in humans would be between 0.0 and 0.81 mg/kg/day. The major target organs of toxicity associated with bis-POM PMEA treatment were liver, kidney, spleen/thymus and bone marrow. A 14-day oral toxicity study in rats was conducted at dose levels of 0, 11, 37 or 110 mg/kg/day. An oral dose of 11 mg/kg/day may be considered the NOEL. Based on the body surface area, an equivalent dose for a 60 kg person would be 1.8 mg/kg/day. Kidney and gastrointestinal tract were identified as the target organs. 4-Week oral toxicity study in Sprague-Dawley rats:

the test compound was administered at dose levels of 0, 12 or 37 mg/kg/day. Kidney was found to be the target organ. A dose level of 4.0 mg/kg/day may be considered the NOEL for this study. With a dose conversion based on body surface area, the equivalent oral dose for humans would be 0.6 mg/kg/day. 6-Month oral toxicity study in Sprague-Dawley rats: adefovir dipivoxil was administered at dose levels of 0, 0.4, 2 or 10 mg/kg/day. The NOEL for bis-POMPMEA in rats following oral gavage administration once daily for up to 26 consecutive weeks was 2 mg/kg/day. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 0.33 mg/kg/day. Kidney was identified as the target organ in both male and female rats. 4-Week toxicity study in cynomolgus monkeys: the test compound was administered at dose levels of 0, 8, 25 or 75 mg/kg/day. A NOEL could not be identified in this study. In monkey, the primary target organs were kidney and stomach. 13-Week oral toxicity study in cynomolgus monkeys: adefovir dipivoxil was administered at dose levels of 0, 1, 5 or 25 mg/kg/day. A dose of 1.0 mg/kg/day may be considered a NOEL for bis-POMPMEA in this study. The primary target organ was kidney. Liver enzyme activity was increased in high dose animals; and it was reversible in four weeks of recovery. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 0.33 mg/kg/day. 52-Week oral toxicity study in cynomolgus monkeys: adefovir dipivoxil was administered at dose levels of 0, 0.2, 1 or 5 mg/kg/day. A dose of 1.0 mg/kg/day may be considered a NOEL for bis-POMPMEA in this study. The primary targets organs were bone marrow and kidney. Liver enzyme activity was increased in high dose animals; it was not completely reversible in the four weeks of recovery period. Serum Carnitine Levels in Monkeys Administered Adefovir dipivoxil for 13 and 52 Weeks in Toxicity Studies: treatment with adefovir dipivoxil at > 1 mg/kg/day for 13 or 52 weeks in cynomolgus monkeys caused decreased total and free carnitine levels; partial resolution of the carnitine deficits occurred in the treated animals relative to the controls after 4-weeks of recovery.

Adefovir dipivoxil: oral (gavage) oncogenicity study in the albino mouse: the oncogenicity potential of adefovir dipivoxil was investigated in mice at dosages of 0, 1, 3 or 10 mg/kg/day in comparison with untreated controls for a period of 104 weeks. Adefovir dipivoxil was not oncogenic in mice. The human AUC at a dose level of 10 mg/day (the proposed clinical dose) was approximately 0.2 µg*hr/ml. The average systemic AUC measured at the high dose level of 10 mg/kg/day during week 26 was 2.62 µg*hr/ml for the mice. Thus, the mouse:human AUC ratio was approximately 13 or drug exposure in the mice was 13-fold than that of the clinical dose.

Adefovir (PMEA):

Multiple-dose toxicology studies-adefovir: groups of Sprague-Dawley rats were administered iv PMEA at dose levels of 0, 10, 100 or 250

mg/kg/day for 5 days. A dose of 10 mg/kg/day of PMEA may be considered the NOAEL; an equivalent dose in humans would be 1.4 mg/kg/day. Adefovir was administered at daily iv dose levels of 0, 1, 10 or 50 mg/kg/day for 2-weeks. A dose of 1.0 mg/kg/day may be considered a NOEL for PMEA in this study. Based on a body surface area factor, an equivalent dose in humans would be 0.16 mg/kg/day. Four-week iv toxicity study in Sprague-Dawley rats: PMEA was injected into rats at dose levels of 0, 1, 5 or 15 mg/kg/day. A dose level of 5.0 mg/kg/day may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 0.81 mg/kg/day. Four-week sc toxicity study in VAF CD rats: sc PMEA was injected into rats at dose levels of 0, 3, 20 or 40 mg/kg/day. The major toxic effects of sc administration of PMEA to rats for 30 days involved the kidneys and hemopoietic system. Nephrotoxicity was manifested by increased serum levels of BUN and creatinine, and decreased serum potassium in mid and high dose animals. The low dose (3 mg/kg/day) may be considered a NOAEL. Based on equivalent body surface area dosage conversion, the highest sc dose for humans would be 0.49 mg/kg/day. Fourteen-day oral toxicity study in cynomolgus monkeys: PMEA was orally administered to monkeys at dose levels of 0, 3, 10 or 30 mg/kg/day. A dose level of 30 mg/kg/day may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent oral dose for humans would be 10 mg/kg/day. One-month iv toxicity study in cynomolgus monkeys: PMEA was administered by iv into monkeys at dose levels of 0, 1, 5 or 25 mg/kg/day. A dose level of 5 mg/kg/day may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 1.66 mg/kg/day. One-month sc toxicity study in cynomolgus monkeys: PMEA was administered subcutaneously into monkeys at dose levels of 0, 3, 20 or 40 mg/kg/day. Daily sc administration of PMEA (3 mg/kg/day) was associated with minimal but detectable changes similar to those seen with greater severity in the animals administered 20 or 40 mg/kg/day. A NOEL could not be identified. Three-month iv or sc toxicity study in cynomolgus monkeys: PMEA was administered via iv or sc routes to monkeys at dose levels of 0, 3, 8 or 20 mg/kg/day. A dose level of 5 mg/kg/day may be considered the NOAEL for this study. With a dose conversion based on body surface area, the equivalent intravenous dose for humans would be 1.66 mg/kg/day. PMEA appeared to preferentially affect the skin. A NOEL could not be determined [less than 3 mg/kg/day]. Kidney and skin were identified as the target organs. In regards to the systemic effects of PMEA, other than those noted in the skin, the NOAEL was 8 mg/kg/day. In a comparison between the iv and the sc routes of administration of 8 mg/kg/day PMEA, there did not appear to be any apparent differences in the incidence or severity of changes seen. Both groups of animals displayed approximately the same level of skin involvement and hyper-pigmentation, and neither group of animals displayed indications of systemic toxicity.

Special toxicology studies-adefovir dipivoxil: dermal sensitization in guinea pigs was evaluated at dose levels of 25%, 50%, 75% or 100% w/v in mineral oil applied to skin sites. The test compound was not considered to be a contact sensitizer in guinea pigs. A primary skin irritation study in rabbits: five male and one female New Zealand White rabbits were applied a 1" * 1" square 4 ply gauze patch containing 0.5 mg of bis-POM PMEA on the dorsal area of the trunk. The test compound was found to be a slight irritant to the skin of the rabbit. A primary eye irritation study in rabbits: New Zealand White rabbits (3/group) received a single 0.047 g (0.1 ml) dose of bis-POM PMEA in the conjunctival sac of the right eye. The test compound was found to be a severe irritant to the ocular tissue of the rabbit without a post-dose saline eye rinse. Bis-POM PMEA should be considered a mild irritant to the ocular tissue of the rabbit following a 30-second post-instillation saline rinse.

Reproductive and developmental toxicity studies-adefovir dipivoxil: oral gavage fertility and general reproduction toxicity study in rats-groups of male and female Sprague-Dawley rats were administered adefovir dipivoxil via gavage at dose levels of 0, 1.2, 6 or 30 mg/kg/day for females beginning 15 days before a 21-day cohabitation period and continuing through day 7 of presumed gestation or the day before scheduled sacrifice and, for males beginning 28 days before the cohabitation period and continuing until sacrifice. Fertility parameters: were unaffected. Female rats: all female rats survived to scheduled sacrifice. Estrous cycling, mating and fertility parameters were unaffected. The NOELs for systemic toxicity in this study was 1.2 mg/kg/day for male and 6 mg/kg/day for female rats. Based on a body surface area conversion factor, equivalent doses in humans would be 0.19 mg/kg/day for males and 0.97 mg/kg/day for females. Based on these data, the NOEL for effects of adefovir dipivoxil on fertility and reproductive performance in male and female rats is 30 mg/kg/day. Based on a body surface area conversion factor, equivalent dose in humans would be 4.87 mg/kg/day. Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of adefovir dipivoxil in rats, including a postnatal behavioral/functional evaluation: groups of presumed pregnant female Sprague-Dawley rats were administered adefovir dipivoxil via gavage at dose levels of 0, 2.5, 10 or 40 mg/kg/day beginning on gestation day (GD) 7 and continuing through gestation day 24 (rats that did not deliver a litter) or lactation day 20 (rats that delivered a litter). F0 generation: body weight gains were significantly reduced in the high dosage group ($p<0.01$) throughout the gestation dosage period (GDs 7-20) and the entire gestation period (GDs 0-20). Natural delivery observations: a significant decreased ($p<0.05$) number of dams (high) delivered on GD 22 and a significantly increased ($p<0.05$) number of dams (high) delivered on GD 23. These differences from the control group values were not considered related to the test article because duration of gestation and the total delivery times did not differ among the groups. F1

generation: average pup weights per litter were significantly ($p < 0.05$) decreased (high). All other litter observations were unaffected. There were no statistically significant or biologically important differences among the four dosages groups in the incidences of clinical or necropsy observations. The lower body weights for the high dose group rats (at weaning), as an indication of the toxicity, was evident throughout the observation period. However, body weight gains were not significantly different from the controls throughout the postweaning period. F2 generation: continued observations of the F1 generation from weaning through mating and Caesarian-delivery of an F2 generation revealed no adverse effects of the test article to the F0 dams. Treatment of the F1 generation rats did not affect litter averages for corpora lutea, implantations, live litter sizes, resorptions, fetal body weight, fetal sex ratios and the number of dams with any resorptions.

The NOELs for maternal and developmental, and reproductive toxicities were 10, and 40 mg/kg/day, respectively. Based on a body surface area conversion factor, equivalent doses in humans for maternal, developmental, and reproductive toxicities would be 1.62, and 6.5 mg/kg/day, respectively. Oral (stomach tube) developmental toxicity study of bis-POMPMEA in Rabbits: groups of timed-pregnant female New Zealand White rabbits were administered orally (via stomach tube) bis-POMPMEA at dose levels of 0, 1, 5 or 20 mg/kg/day once daily on day 6-18 of presumed gestation. No maternal or fetal toxicity resulted from administration of bis-POMPMEA to pregnant New Zealand White rabbits. Average maternal body weight changes, body weights and absolute (g/day) and relative (g/kg/day) feed consumption values were unaffected; there were no statistically or biologically important differences among the four dosage groups. Caesarian-sectioning and litter observations were unaffected by the test article. The litter averages for corpora lutea, implantations, live and dead fetuses were comparable among the four dosage groups and did not differ significantly. No fetal external, soft tissue or skeletal malformations or variations were attributed to administration of the test article. A dosage of 20 mg/kg/day may be considered the maternal and fetal NOELs. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 6.45 mg/kg/day. A developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of PMEA administered intravenously to presumed pregnant rats: groups of presumed pregnant rats were administered PMEA intravenously once daily at dose levels of 0, 2.5, 10 or 20 mg/kg/day during gestation days 6 through 15 to evaluate developmental toxicity and teratogenic potential of the test compound. Treatment-related embryotoxicity and fetal malformations were observed at PMEA dosages of > 10 mg/kg/day (mid and high). Fetal malformation (incompletely ossified pubes) also occurred in low dose animals. Treatment-associated embryotoxicity and teratogenicity were observed at PMEA dosages of > 10 mg/kg/day but the dosages also resulted in significant maternal toxicity. Therefore, based on the results of this study, PMEA can not be

categorized unequivocally as a teratogen because maternal toxicity was also seen at the same dose levels. The developmental NOEL for PMEA is 10 mg/kg/day since there were effects on live fetal weights at the low dose males. On the basis of a body surface area conversion factor, an equivalent dose in humans would be 1.62 mg/kg/day. The NOEL dose is close to the human dose.

Genotoxicology studies-adefovir dipivoxil and adefovir: Mutagenicity test on bis-POM PMEA in the in vivo mouse micronucleus assay: Male and female CD-1 mice were administered adefovir dipivoxil via oral gavage at dose levels of 0, 500, 1000 or 2000 mg/kg/day (high) to evaluate clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in bone marrow. Bis-POM PMEA was found to be cytotoxic and clastogenic in the bone marrow in the mouse micronucleus assay. Mutagenicity test on bis-POM PMEA in the L5178Y $TK^{+/-}$ mouse lymphoma mutation assay:
bis-POM PMEA was tested in the L5178Y $TK^{+/-}$ mouse lymphoma cell line at concentrations of 1.57, 3.13, 6.25, 12.5 37.5 or 50.0 µg/ml in the presence or absence of rat liver S9 metabolic activation to evaluate the ability of bis-POM PMEA to induce forward mutations at the thymidine kinase locus. Bis-POM PMEA was found to be a positive mutagen in the presence or absence of metabolic activation. Ames Microbial Mutagenicity Assay and E. Coli WP2 uvrA Reverse Mutation Assay (PMEA) The compound was tested with and without exogenous metabolic activation using the S-9 fraction of a rat liver homogenate in the mutation assay at five nominal concentrations (312.5, 625, 1250, 2500 and 5000 µg/plate. PMEA was not mutagenic in the Ames microbial mutagenicity assay or the E. coli reverse mutation assay up to a concentration of 5000 µg/plate. Human Peripheral Blood Lymphocyte Clastogenesis Assay (PMEA) PMEA exhibited a statistically significant clastogenic effect in the human peripheral blood lymphocyte chromosome aberration assay at the 25 and 50 µg/ml level tested. PMEA proved to be more clastogenic than the reference agent, ganciclovir.

Safety pharmacology studies-adefovir dipivoxil: Effects of bis-POM PMEA in the Irwin Test in the mouse. Groups of male CD-1 mice were dosed via oral gavage at a single dose of bis-POM PMEA at dose levels of 0, 10, 30 or 100 mg/kg (high) to detect gross behavioral and physiological states. With the exception of pilo-erection, no other drug-related gross behavioral or physiological changes were observed in the Irwin test in the mice: Effects of bis-POM PMEA on renal function in saline-loaded rats. Groups of male Sprague-Dawley rats were dosed via oral gavage at a single dose of bis-POM PMEA at dose levels of 0, 10, 30 or 100 mg/kg to assess the effects of bis-POM PMEA on urine pH, urine volume and urinary excretion of sodium, potassium and chloride in rats. A decrease in urine volume and potassium excretion was seen in rats (mid). In the high dose group, there were significant decreases in electrolyte excretion and urine

volume compared to the controls. Over the 3-6 hr postdose period, bis-POM PMEA caused a significant decrease in urine volume (high) compared to the controls. Over the 6-24 hr postdose period, urinary excretions of potassium and chloride were significantly decreased in rats (high). Effects of bis-POM PMEA on gastrointestinal transit of a charcoal meal in rats: Groups of male Sprague-Dawley rats were dosed via oral gavage at a single dose of bis-POM PMEA at dose levels of 0, 10, 30 or 100 mg/kg to assess the effects of bis-POM PMEA on the gastrointestinal transit of a charcoal meal in rats. A dose of 10 mg/kg bis-POM PMEA may be considered a NOEL. Based on a body surface area conversion factor, an equivalent dose in humans would be approximately 1.62 mg/kg. Cardiovascular and respiratory evaluation in the anaesthetized dog following intraduodenal administration: Female beagle dogs were anesthetized by an iv injection of sodium thiopentone and maintained intravenously by a mixture of α-chloralose/and pentobarbitone sodium to examine the cardiovascular and respiratory effects of a vehicle and adefovir dipivoxil following a single intraduodenal administration at dose levels of 0, 4 or 12 mg/kg. Adefovir dipivoxil had no overt effects on any of the cardiovascular or respiratory parameters at either of the doses tested.

Pharmacokinetic and ADME studies-adefovir dipivoxil: adefovir has low oral bioavailability in animals and humans, apparently due to limited intestinal permeability. Adefovir dipivoxil was designed as a lipophilic prodrug of adefovir to provide intestinal permeability and greater bioavailability. Adefovir dipivoxil showed a 10-fold greater permeability across human intestinal cells in vitro compared to adefovir. Following absorption, adefovir dipivoxil was rapidly converted to adefovir. No metabolites related to the purine nucleus of adefovir dipivoxil (including the monoester) were detected in the plasma or urine in animals or humans following oral administration of adefovir dipivoxil. The oral bioavailability of adefovir from a single oral dose of adefovir dipivoxil was 40-45% in mice, 13-43% in rats, 35-46% in dogs and 14-32% in monkeys. The oral bioavailability in dogs was independent of the formulation, feeding state or the pH of the intestinal tract. In humans, the oral bioavailability of adefovir (25-30%) from a single oral dose of adefovir dipivoxil was independent of formulation (suspension or tablet) when administered in fasted state and 41% in the presence of food. The pharmacokinetics of adefovir in plasma following oral administration of adefovir dipivoxil were independent of dose. Maximum concentrations of adefovir in the systemic circulation occurred within 1-2 hr in most species and were proportion to dose. Plasma adefovir levels were 10- to 20-fold higher than those produced by equivalent oral dose adefovir. The apparent oral clearance of adefovir following administration of adefovir dipivoxil was similar in mice, rats and monkeys (approximately 3-6 l/hr/kg), but was lower in dogs (0.7-0.9 l/hr/kg). Adefovir was excreted by renal elimination without further metabolic changes. The terminal half-life of elimination of adefovir ranged from 3-5

hr in rats to 7-10 hr in dogs. In tissue distribution studies with radiolabeled adefovir dipivoxil in rats and monkeys, highest concentrations of radioactivity were detected in the intestinal tissues, kidneys and liver. Recovery of radioactivity in urine was generally consistent with oral bioavailability. There was no evidence of a significant change in the pharmacokinetics of adefovir on repeated daily dosing of adefovir dipivoxil for up to 13-weeks in mice, 26-weeks in rats or 52-weeks in monkeys. Adefovir dipivoxil was not a substrate for cytochrome P450 and it does not inhibit the major isoforms of cytochrome P450 (including CYP3A4). Repeated dosing of adefovir dipivoxil had no effect on the activity of hepatic cytochrome P450 in rats. Due to the rapid hydrolysis (half-life, 3 min) of adefovir dipivoxil in plasma and serum, protein binding was not investigated in animal species; binding of adefovir to human plasma or serum proteins was negligible (<2%).

Pharmacokinetic and ADME studies-adefovir: the pharmacokinetics of adefovir were examined in mice, rats, dogs and monkeys. Following iv administration of adefovir in animals, the pharmacokinetics of adefovir were generally characterized by a bi-exponential decline in plasma levels and clearance at a rate exceeding glomerular filtration. The volume of distribution suggested that the drug was distributed in total body water. Tissue distribution studies with unlabelled or radiolabeled adefovir in mice, rats and monkeys, showed that adefovir was eliminated unchanged in the urine (approximately 64-83%) and the highest tissue concentrations were achieved in the kidneys of all species. Adefovir was eliminated mainly by renal excretion and by a combination of glomerular filtration and active renal tubular secretion. No metabolites of adefovir were observed in plasma, urine or tissues of rats or monkeys. Adefovir monophosphate (PMEAp) was observed in extracts of kidney and liver of mice. The low oral bioavailability of adefovir was demonstrated in mice (16%), rats (11%) rhesus (>1%) and cynomolgus (4%) monkeys.

III. Administrative

A. Reviewer singnature:_____

B. Supervisor signature: Concurrences:

HFD-530/JFarrelly
HFD-530/PVerma

Disk
HFD-530/JFarrelly

I. INTRODUCTION AND DRUG HISTORY:

Adefovir dipivoxil (bis-POM PMEA) is an oral prodrug of PMEA (adefovir). PMEA, the parent compound, is a nucleotide analog, which belongs to a class of compounds termed phosphonomethylether nucleotide analogues. PMEA and bis-POM PMEA have been shown to have anti-viral activity against a broad spectrum of retro- and herpes viruses including pathogens such as human immunodeficiency virus type 1 and 2, cytomegalovirus, herpes simplex virus type 1/2, Rauscher murine leukemia virus, Moloney murine sarcoma virus, feline leukemia, simian immunodeficiency virus and Epstein-Barr virus. The intracellular active metabolite of both the compounds is PMEA diphosphate (PMEApp), which is found to be an inhibitor of HBV polymerase, with respect to the natural substrate dATP and functions as a chain terminator of HBV DNA replication. This NDA is for adefovir dipivoxil as a competitive inhibitor for the treatment of HBV infection.

Previously under NDA numbers [redacted] and 21-138, adefovir dipivoxil has been a subject of clinical investigation for the treatment of HIV infection, where the test compound was being administered as an oral formulation (120 mg, $AUCss_{0-\infty}$ = 1.68 μg*hr/ml). In this application, the test article is being evaluated at a dose level of 10 mg tablet ($AUCss_{0-\infty}$ = 0.210 μg*hr/ml).

The safety profile of adefovir dipivoxil has been extensively evaluated in toxicology, Pharmacokinetic/ADME and pharmacology studies using the test compound. This evaluation included studies in mice, rats, woodchucks, guinea pigs, rabbits, dogs and monkeys. The principal target organs of toxicity following oral administration of adefovir dipivoxil or intravenous/subcutaneous administration of adefovir in animal models were the kidneys, gastrointestinal tract, liver and lympho-proliferative tissues (including bone marrow). Nephrotoxicity was the primary dose-limiting toxicity associated with the oral administration of adefovir dipivoxil in rats and monkeys. Liver and lympho-proliferative tissues were the most sensitive target organs in mice. In all species tested, adefovir dipivoxil was hydrolyzed to adefovir following absorption and adefovir was cleared exclusively by renal elimination, without further metabolic changes. The elimination of adefovir occurred by a combination of glomerular filtration and tubular secretion.

In humans overall, the nature and frequency of clinical adverse events during the first 48 weeks of the pooled studies were similar between the adefovir dipivoxil 10 mg and placebo groups. The most commonly reported adverse events in these groups were: headache, pharyngitis, asthenia, abdominal pain and flu syndrome.

Among patients in the all adefovir dipivoxil 10 mg group with up to 96 weeks of treatment, the frequency for these events was comparable or lower than the 48 week adefovir dipivoxil 10 mg and placebo groups.

II. SAFETY PHARMACOLOGY:

Safety pharmacology studies summary: The studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practice Regulations.

1. **Effects of bis-POM PMEA in the Irwin Test in mouse, Lot # 2166-A9P, Gilead Sciences, Foster City, CA, March 12, 1998, (GS-98-TOX-0840-001)***

2. **Effects of bis-POM PMEA on renal function in saline-loaded rats, Lot # 2166-A9P, Gilead Sciences, Foster City, CA, March 17, 1998, (GS-98-TOX-0840-002)***

3. **Effects of bis-POM PMEA on gastrointestinal transit of a charcoal meal in rats, Lot # 2166-A9P, Gilead Sciences, Foster City, CA, March 13, 1998, (GS-98-TOX-0840-003)***

4. **Adefovir dipivoxil: Cardiovascular and respiratory evaluation in the anaesthetized dog following intraduodenal administration, Lot # 2166-A-9P, Gilead Sciences, Foster City, CA, August 19, 1998, (98-TOX-0840-004)***

Review of safety pharmacology studies:

1. Effects of bis-POM PMEA in the Irwin Test in mouse, Lot # 2166-A9P, Gilead Sciences, Foster City, CA, March 12, 1998, (GS-98-TOX-0840-001)*

Groups of male CD-1 mice (6 mice/group) were dosed via oral gavage at a single dose of bis-POM PMEA at dose levels of 0 (vehicle control), 10 (low), 30 (mid) or 100 mg/kg (high) to detect gross behavioral and physiological state of mice. Clonidine hydrochloride was used as a reference substance. The parameters defined in the Irwin test (abnormal gait, carriage, aggressiveness, apathy, alertness, body tone, catalepsy, exophthalmia, fearfulness, fighting, grip strength, pain response, locomotor activity, pilo-erection, ptosis, pupil diameter, respiration, restlessness, salivation, touch response, tremor, twitches, vocalization and stereotype behavior) were systematically evaluated for each mouse at 0.5, 1, 3 and 6 hr after dosing. Results: pilo-erection (4/6 mice at 0.5 hr postdose

and 2/6 mice at 1 and 3 hr postdose) and increased pupil diameter (2/6 mice at 0.5 hr and 3/6 mice at 1 hr post dose were seen in the mid dose group. Pilo-erection was also observed in the high dose group (3/6 mice at 0.5 hr postdose. Conclusions: with the exception of pilo-erection, no other drug-related gross behavioral or physiological changes were observed in the Irwin test in the mice.

2. Effects of bis-POM PMEA on renal function in saline-loaded rats, Lot # 2166-A9P, Gilead Sciences, Foster City, CA, March 17, 1998, (GS-98-TOX-0840-002)*

Groups of male Sprague-Dawley rats (8 rats/group) were dosed via oral gavage at a single dose of bis-POM PMEA at dose levels of 0 (vehicle control), 10 (low), 30 (mid) or 100 mg/kg (high) to assess the effects of bis-POM PMEA on urine pH, urine volume and urinary excretion of sodium, potassium and chloride in rats. Furosemide was used as a reference substance. Urine samples were collected at 3, 6 and 24 hr postdose. Results: over the 0-3-hr postdose period, bis-POM PMEA (low) had no significant effects on urine volume or electrolyte excretion compared to the vehicle controls. A decrease in urine volume and potassium excretion was seen in rats (mid). In the high dose group, there were significant decreases in electrolyte excretion and urine volume compared to the controls. Over the 3-6 hr postdose period, bis-POM PMEA caused a significant decrease in urine volume (high) compared to the controls. Over the 6-24 hr postdose period, urinary excretions of potassium and chloride were significantly decreased in rats (high). Furosemide affected renal function in a manner consistent with its pharmacological classification as a diuretic agent.

3. Effects of bis-POM PMEA on gastrointestinal transit of a charcoal meal in rats, Lot # 2166-A9P, Gilead Sciences, Foster City, CA, March 13, 1998, (GS-98-TOX-0840-003)*

Groups of male Sprague-Dawley rats (8 rats/group) were dosed via oral gavage at a single dose of bis-POM PMEA at dose levels of 0 (vehicle control), 10 (low), 30 (mid) or 100 mg/kg (high) to assess the effects of bis-POM PMEA on the gastrointestinal transit of a charcoal meal in rats. Morphine hydrochloride was used as a reference substance. Approximately 30 min after dosing, each animal was given a 1 ml suspension of charcoal orally. A further 30 min after being given the charcoal meal, each rat was killed by cervical dislocation and the intestine was exposed. The distance that the charcoal meal had traveled along the intestine from the pyloric sphincter was measured. The stomach was weighed at the end of the experiment to give an estimate of the weight of the stomach plus contents and an indication of the degree of gastric emptying. Results: gastrointestinal transit-the mean

values for the percent distance traveled along the intestine by the charcoal meal were 55.6% (vehicle controls) 49.8% (low), 52.3% (mid) and 48% (high), the treated values were not significantly different from the vehicle controls. Gastric Emptying: there was a significant increase in stomach weight (mid or high) compared to the controls. It was noted that the stomachs of the animals were fluid filled. Conclusions: under the conditions of the study, a dose of 10 mg/kg bis-POM PMEA may be considered a NOEL. Based on a body surface area conversion factor, an equivalent dose in humans would be approximately 1.62 mg/kg.

4. Adefovir dipivoxil: Cardiovascular and respiratory evaluation in the anaesthetized dog following intraduodenal administration, Lot # 2166-A-9P, Gilead Sciences, Foster City, CA, August 19, 1998, (98-TOX-0840-004)*

A total of three female beagle dogs were anesthetized by an iv injection of sodium thiopentone and maintained intravenously by a mixture of α-chloralose/and pentobarbitone sodium to examine the cardiovascular and respiratory effects of a vehicle and adefovir dipivoxil following a single intraduodenal administration at dose levels of 0 (50 mM citric acid, vehicle), 4 or 12 mg/kg. The trachea was cannulated and the cannula connected to a pneumatic screen to allow measurement of respiration tidal volume and the derived parameters of respiration rate and minute volume. All the physiological parameters were monitored continuously for a period of 30 min prior and then 2 hr following the treatment. Results: in one dog (# 2490; vehicle, 4 and 12 mg/kg), there was a slight transient increase in mean blood pressure observed approximately 5 min (vehicle) and 1 min (4 and 12 mg/kg); a decrease in heart rate (4 mg/kg) and a transient increase in heart rate (12 mg/kg); a transient decrease in left ventricular dp/dt (vehicle) and a transient increase in left ventricular systolic pressure accompanied by a concurrent increase in left ventricular dp/dt maximum (4 mg/kg); an increase in mean femoral resistance (vehicle, 4 and 12 mg/kg) and a decrease in respiration rate and an increase in tidal volume after the treatment.

In the other two animals, intraduodenal administration of vehicle or adefovir dipivoxil (4 and 12 mg/kg) did not induce marked or sustained effects on any of the cardiovascular or respiratory parameters. The EEG remained unaffected by vehicle and adefovir dipivoxil treatment in all three dogs. Conclusions: intraduodenal administration of vehicle or adefovir dipivoxil had no overt effects on any of the cardiovascular or respiratory parameters at either of the doses tested.

III. PHARMACOKINETICS/TOXICOKINETICS:

Pharmacokinetics/toxicokinetics studies summary:

1. Effect of oral administration of adefovir dipivoxil on hepatic cytochrome P-450 concentration and associated drug metabolizing activities in the rat, December 30, 1998, (98-DDM-0840-002)

2. Pharmacokinetic report of adefovir from a 13-week oral gavage toxicity study of bis-POM PMEA in the albino mouse, Lot # 2166-A6P,

3. Determination of Oral Bioavailability of — 40085 (PMEA) when Administered as Prodrugs, Lot # 29087-025, August 6, 1991,

4. PMEA, POMPMEA, and bis-POMPMEA in Plasma Following Oral Administration of bis-POMPMEA to Rats, Lot # C90G741, Gilead Science, Foster City, CA, January 14, 1994, (DDM-GE-011494/HWI -6511-101)

5. Oral Bioavailability of PMEA from PMEA Prodrugs in Male Sprague-Dawley Rats, Lot # 547-95-21, Gilead Science, Foster City, CA, January 17, 1994, (DDM-JPS-120693)

6. Tissue distribution of ^{14}C-labelled bis-POMPMEA in Sprague-Dawley rats, Lot # DB579-71, August 5, 1994, — GSI-R77-94-6/2R77/93-DDM-0840-004/P0840-00005)

7. Analysis of data from — study 2-R77: tissue distribution of ^{14}C in Sprague-Dawley rats, February 1, 1995, (2-R77/93-DDM-0840-004/P0840-00011)

8. Pharmacokinetics of PMEA in a 14-day Repeated Dose Oral Toxicity Study with bis-POMPMEA in Rats (A Report on Analysis of Data from Toxicity Study HWI-6511-104/Gilead Study No. 94-TOX-0840-002-BA)

9. Pharmacokinetics of bis-POM PMEA in a 6-Month chronic oral toxicity study of bis-POM PMEA in Rats, Lot # 808-GS-93, . January 20, 1998, (94-TOX-0840-003-PK\Project No. 86539)

10. Pharmacokinetics of adefovir in an oral carcinogenicity study of adefovir dipivoxil in the rat (a report of

analysis of data from study 96-TOX-0940-003, January 25, 1999, (96-TOX-0840-003-PK)

11. Determination of adefovir dipivoxil in hepatic portal blood following oral administration to rats, January 6, 1999, (97-DDM-0840-002)

12. Concentration of adefovir in plasma at 2 hours following an oral dose of adefovir dipivoxil in a 14-day oral gavage toxicity study of bis-POM PMEA in the albino rat, Lot # TX840-97-05, [redacted] December 30, 1998, (97-TOX-0840-006-PK)

13. Pharmacokinetics of PMEA in rats from a developmental toxicity study of PMEA administered intravenously to presumed pregnant rats (A report of analysis of plasma concentration data from toxicity study # 94-TOX-0393-001), 16 December, 1998, (94-TOX-0393-001-PK)

14. Oral bioavailability of Adefovir (PMEA) from bis-POM PMEA granules in woodchucks (97-DDM-0840-001)

15. Bioavailability of PMEA from Three Oral Formulations of the Prodrug bis-POMPMEA in Fasted Male Cynomolgus Monkeys, Lot # 477-24-33, Gilead Science, Foster City, CA, May 19, 1993, (DDM-KC-042793)

16. An Open Balance Study of IV ^{14}C-PMEA and Oral ^{14}C-bis-POMPMEA Administered to Cynomolgus Monkeys, Lot # JPS-646-9/11, [redacted] December 22, 1993, ([redacted]-GSI-R23-93-190/93-DDM-0840-003)

17. Bioavailability of PMEA from a Prototype Oral Suspension Formulation of the Prodrug bis-POMPMEA in Fasted Male Cynomolgus Monkeys, Lot # 840J93-01, Gilead Science, Foster City, CA, January 3, 1994, (DDM-JPS-121593)

18. Bis-POMPMEA concentrations from a 4-week oral toxicity study with bis-POMPMEA in Cynomolgus monkeys, Lot # JM-614-5, [redacted] '6511-100/93-TOX-0840-002-BA)

19. Pharmacokinetics of PMEA in a 91-day Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # JM-614-70-11, [redacted] September 1, 1994, ([redacted]/6511-103/94-TOX-0840-001-BA/P0840-00008)

20. Pharmacokinetics of bis-POM PMEA in a 52-Week Oral

Toxicity Study with bis-POM PMEA in Cynomolgus Monkeys, Lot # TX840-96-01, January 23, 1998, (T0840-00019/95-TOX-0840-006-PK)

21. Protein Binding of Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum, Cidofovir Lot # 1966-C-9P), Gilead Sciences, Inc., Foster City, CA, June 9, 1995 (PO504-00039/95-DDM-XXXX-001)

22. A Pilot Study of Bioavailability in the Rat, 40085 (PMEA), Lot # 26870-059 and 26614-069a, April 12, 1989, (-25365)

23. Pharmacokinetics, Mass Balance and Tissue Distribution of Radiolabelled ^{14}C-PMEA, Lot # NB 316-95-02 and NB 376-14-9-01, August 20, 1992, (830-GS-001-92)

24. Bioequivalency study of ^{14}C labeled GS0393 after administration to Cynomolgus Monkeys, Lot # NB316-95-50, December 17, 1992, (2-K03/ -GSI-K03-92-111)*

25. Pharmacokinetics of adefovir in a ninety-one day repeated dose intravenous or subcutaneous toxicity study of adefovir administered to cynomolgus monkeys, February 23, 1999, (93-TOX-0393-001-PK)

26. Pharmacokinetics of adefovir in pregnant rats and rabbits after iv administration of adefovir or oral administration of adefovir dipivoxil, 27 March, 1998 (96-TOX-0840-002-PK)

27. Bioavailability of PMEA from two clinical formulations of the Prodrug bis-POMPMEA in beagle dogs, Gilead Science, Foster City, CA, March 7, 1995, (94-DDM-0840-002)

28. Bioavailability of PMEA from tablet formulations of the Prodrug bis-POMPMEA in beagle dogs, Gilead Science, Foster City, CA, March 26, 1998, (94-DDM-0840-004)

29. Bioavailability of PMEA from various formulations of the Prodrug bis-POMPMEA in monkeys, Gilead Science, Foster City, CA, May 13, 1994, (DDM-JPS-020394)

30. Bioavailability of PMEA from the Prodrug bis-POMPMEA in monkeys, Gilead Science, Foster City, CA, May 13, 1994, (DDM-JPS-020394)

31. Bioavailability of PMEA from the Prodrug bis-POMPMEA in monkeys; bis-POMPMEA Sulfate, and a clinical tablet formulation of bis-POMPMEA, Gilead Science, Foster City, CA, March 6, 1998, (94-DDM-0840-001B)

32. Urinary excretion of ^{14}C-adefovir following intravenous administration to Sprague-Dawley rats, 19 March, 1998 (94-DDM-0393-004)

33. toxicokinetics study of adefovir dipivoxil in presumed pregnant rats and rabbits, Lot # 01-167-DK, Gilead Science, Inc., Foster City, CA, April 16, 1997, (96-TOX-0840-002/ML-GSI-3D1096-225)

Review of pharmacokinetics/toxicokinetics studies:

1. Effect of oral administration of adefovir dipivoxil on hepatic cytochrome P-450 concentration and associated drug metabolizing activities in the rat, December 30, 1998, (98-DDM-0840-002)

A group of male Sprague-Dawley derived rats (4) received daily oral doses of adefovir dipivoxil (100 mg/kg/day for 3 days) to study the potential for adefovir dipivoxil to induce hepatic cytochrome P-450 activity. On day 4, the animals were sacrificed and livers were removed. Hepatic microsomes were prepared by standard methods, The concentration of cytochrome P-450 was determined by the carbon monoxide binding method. The drug metabolizing activity of the liver microsomes was determined by incubation with model substrates including 7-ethoxycoumarin, benzphetamine, erythromycin and dextromethorphan. Results: adefovir dipivoxil administration had no effect on liver weight or total hepatic microsomal cytochrome-450 concentration. Adefovir dipivoxil administration did not affect the activities of cytochrome-450 isomers involved in 7-ethoxycoumarin O-deethylation, benzphetamine N-demethylation, erythromycin N-demethylation and detromethorphan O-demethylation. Conclusions: the oral administration of adefovir dipivoxil did not affect the hepatic cytochrome-450 system in the rat.

2. Pharmacokinetic report of adefovir from a 13-week oral gavage toxicity study of bis-POM PMEA in the albino mouse, Lot # 2166-A6P, December 31, 1998, (97-TOX-0840-002-PK/T0840-00020)

Groups of male and female CD-1 mice [Crl:CD-1(ICR)BR; 15 animals/group/sex] were dosed daily via oral gavage at dose levels of 0 (vehicle control), 10 (low), 30 (mid) or 100 mg/kg/day (high) for 13 consecutive weeks. The toxicokinetic cohort consisted of 32 male mice (low and mid), and 32 male and 32 female mice (high). Blood samples were taken at predose, 0.5,

1, 2, 3, 4, 8 and 24 hr postdose on the first days of weeks 1 and 13. Concentration of bis-POM PMEA in plasma was determined using

[redacted]

3. Determination of Oral Bioavailability of [redacted] 40085 (PMEA) when Administered as Prodrugs, Lot # 29087-025, [redacted] August 6, 1991, ([redacted]-25804)

Groups of adult male rats (strain: HSD:SD; weight: 140 - 320 g; 3 animals/group; fasted overnight) were administered various prodrugs between 23.74 and 42.79 mg/kg equivalents of PMEA orally at a volume of 10 ml/kg using a 3 inch 20 gauge feeding needle. Two urine samples were collected per rat, a 0 - 24 and 24 - 48 hr sample. PMEA was quantified in the urine by a validated [redacted] method. Purpose of this study was to determine bioavailability of various PMEA prodrugs. A summary of the absolute bioavailability for PMEA after oral administration of the prodrugs of PMEA is shown in Table 2. The absolute bioavailability of PMEA was 7.8%. The group of prodrugs showing the greatest bioavailability values was carbonyl esters; and within that group, the di-

pivaloyloxymethyl ester was the highest (17.3%). This study has shown that, for PMEA, the bioavailability can be enhanced by the oral administration of selected prodrugs.

Table 2
Summary of Bioavailability of PMEA and its Prodrugs

S.N.	Number	Compound Name	F (%)
1	40085	PMEA	7.8
		ALKYL ESTERS	
1	40127	di-ethyl	42.1*
2	43047	di-isopropyl	44.4*
3	43072	mono-isopropyl	5.0
4	43141	mono-octyl	0.8
5	44174	di-trichloroethyl	10.6
6	44175	mono-trichloroethyl	2.5
		ADENINE ANALOGS	
1	43765	mono-isopropyl hypoxanthine	0.0
2	43766	di-isopropyl chloropurine	0.1
		AMIDES	
1	43270	di-methyl propyl phosphonamide	10.6
2	43387	di-methyl cyclic propane amide	5.8
		CARBONYL ESTERS	
1	43769	mono-acetic acid	16.2
2	45254	di-pivaloyloxymethyl	17.3
3	45963	ethyl pivaloyloxymethyl	34.9*
4	45964	2-pivaloyloxymethyl	6.5
		AROMATIC ESTERS	
1	43980	mono-phenyl	14.0
2	43982	di-phenyl	11.1

*** Appeared in the urine as the prodrug**

Comments: The absolute bioavailability of PMEA is relatively low (11% in the previous and 7.8% in present studies). To overcome the problem of low oral bioavailability of PMEA, which could be

attributed to poor absorption, the sponsor has synthesized and tested various prodrugs to enhance the absorption of PMEA and thereby enhancing its oral bioavailability. These prodrugs contain different functional groups attached to the phosphonate moiety, which upon hydrolysis will liberate PMEA. Several of the compounds, after their absorptions, are not able to hydrolyzed to PMEA. Thus they can not be used as prodrugs. However, the oral bioavailability of PMEA can be enhanced up to 17.3% by using selected carbonyl esters of PMEA.

4. PMEA, POMPMEA, and bis-POMPMEA in Plasma Following Oral Administration of bis-POMPMEA to Rats, Lot # C90G741, Gilead Science, Foster City, CA, January 14, 1994, (DDM-GE-011494/ - 6511-101)

Plasma concentrations of PMEA, mono-POMPMEA and bis-POMPMEA were determined following an oral administration of bis-POMPMEA to rats in a safety study ('6511-101/93-TOX-0840-001). Drug was administered by oral gavage once daily at dose levels of 0 (vehicle control), 4 (low), 12 (mid) or 37 mg/kg (high) for a period of 28 days. Plasma samples were obtained at 0.5, 1, 2, 4, 6 and 12 hr post administration on days 1 and 30 from all dose groups. The plasma samples were analyzed by a validated bioanalytical method for PMEA, mono-POMPMEA and bis-POMPMEA. The lower limit of quantitation was for PMEA and for mono-POMPMEA and bis-POMPMEA. No mono-POMPMEA or bis-POMPMEA were detected in any of the sample analyzed. No animal (control and low) showed any quantifiable plasma levels of PMEA. Two animals (mid) had quantifiable plasma levels with a C_{max} of 0.296 µg/ml and T_{max} of 1 hr. At an oral dose of 37 mg/kg/day (high), the observed C_{max} was 0.989 µg/ml, T_{max} was 2 hr, terminal elimination half-life was 4.0 hr and $AUC_{0-\infty}$ was 6.321 µg*hr/ml.

Comments: AUC values for PMEA were available only at the high dose. The comparison of the maximum plasma concentrations indicates dose proportionality between mid and the high dose groups.

5. Oral Bioavailability of PMEA from PMEA Prodrugs in Male Sprague-Dawley Rats, Lot # 547-95-21, Gilead Science, Foster City, CA, January 17, 1994, (DDM-JPS-120693)

The oral bioavailability of PMEA was determined for three PMEA prodrugs formulated as solutions (PEG 400) in male rats. The three prodrugs were bis-POMPMEA, bis-(phenyl)PMEA and bis(o-ethoxyphenyl)PMEA. Plasma samples were obtained over the course of 12 hr and concentrations of PMEA were determined by a validated method. PMEA plasma levels observed following the administration of oral formulations of PMEA prodrugs were

compared to levels observed for an iv PMEA (10 mg/kg) administration. The observed oral bioavailability of PMEA for bis-POMPMEA, bis(phenyl)PMEA and bis(o-ethoxyphenyl)PMEA were 42.6%, 2.8% and 44.3%, respectively.

6. Tissue distribution of ^{14}C-labelled bis-POMPMEA in Sprague-Dawley rats, Lot # DB579-71, [redacted] August 5, 1994, ([redacted]-GSI-R77-94-6/2R77/93-DDM-0840-004/P0840-00005)

A group of 8 rats (4/sex) were dosed orally via gavage with a single dose level of ^{14}C-bis-POMPMEA (10 mg-equiv PMEA/kg; 50 µCi/kg) to study the tissue distribution of the test compound. Each animal was observed at 1, 4 and 6 hr post-treatment during the study for any clinical signs. Results: no abnormalities of any kind were observed during the study period. At 6 hr post-treatment period, in both the male and female rats, the greatest percentage of the recovered ^{14}C activity, an average total of 16.4% in the males and 19.7% in the females, was observed in the three sections of the intestine: duodenum, ileum and jejunum.

Comments: No feces samples were produced by any animal and only one female rat produced a voided urine sample for analysis. Stomach and intestinal contents may have contained additional unabsorbed ^{14}C activity which would have been recovered if the feces samples were produced in the study.

7. Analysis of data from [redacted] study 2-R77: tissue distribution of ^{14}C in Sprague-Dawley rats, February 1, 1995, (2-R77/93-DDM-0840-004/P0840-00011)

A group of 8 rats (4/sex) were dosed orally via gavage with a single dose level of ^{14}C-bis-POMPMEA (10 mg-equiv PMEA/kg; 50 µCi/kg) to study the tissue distribution of the test compound. At 6 hr after the dosing, all animals were sacrificed and tissues removed for analysis. Total radioactivity in tissues was determined by oxidation and liquid scintillation. Results: total recovery (mean ± SD) of the radioactive dose was 24.1 ± 5.0% in males and 29.7 ± 8.8% in females. The results are summarized in Table 3. No intact bis-POMPMEA, mono-POMPMEA or phosphorylated metabolites of PMEA were detected in plasma, blood, urine or in tissue samples. There was no significant difference in the tissue distribution of drug in male and female rats.

Table 3

Distribution of Radioactivity in Tissues at 6 Hour After Oral Administration of ^{14}C-bis-POMPMEA in Rats

Tissues	% Dose Recovered in Tissues		Concentration in Tissues (µg-equiv PMEA/g)	
	Male	Female	Male	Female
Stomach	0.49	0.45	6.0	4.5
Jejunum	7.32	6.81	65.5	54.8
Duodenum	3.82	7.75	36.6	50.3
Ileum	5.29	5.19	36.5	39.8
Cecum	2.15	1.52	35.9	26.5
Colon	0.13	0.34	2.8	7.3
Rectum	0.07	0.23	1.3	3.5
Kidney	1.27	1.64	11.9	15.4
Liver	0.78	0.58	2.1	1.5
Skeletal muscle	0.90	2.51	0.4	0.6
Plasma	0.08	0.07	0.27	0.09
Blood	0.07	0.07	0.14	0.06

Comments: Data from the present study were compared with historical data from a similar study of intravenous ^{14}C-PMEA in rats. Oral administration of an equivalent dose of bis-POMPMEA gave approximately 60-fold higher levels of radioactivity in intestinal tissues than following the intravenous administration of PMEA.

8. Pharmacokinetics of PMEA in a 14-day Repeated Dose Oral Toxicity Study with bis-POMPMEA in Rats (A Report on Analysis of Data from Toxicity Study — 6511-104/Gilead Study No. 94-TOX-0840-002-BA)

Groups of male and female rats were administered bis-POMPMEA via oral gavage at dose levels of 0 (vehicle control), 12 or 37 mg/kg/day in a suspension formulation for 14 consecutive days. Plasma samples were obtained at 0, 1, 2, 4, 6 and 12 hr post-dose on day 14 of the study. The samples were analyzed by — using a validated bioanalytical method in compliance with the GLP regulations. Results: mean pharmacokinetic parameters obtained from rats on day 14 are shown in Table 4.

Table 4

Mean Pharmacokinetic Parameters for PMEA on Day 14 Following Repeated Oral Administration of Bis-POMPMEA to Rats

Parameter	Dose	
	12 mg/kg/day	37 mg/kg/day
$AUC_{0-\infty}$ (µg*hr/ml)	1.83	10.92
Cmax (µg/ml)	0.34	1.24
Tmax (hr)	1.0	1.0
$T_{½}$ (hr)	5.3	5.9
F* (%)	40.4	78.2

* Historical data for iv PMEA in rats demonstrated an $AUC_{0-\infty}$ value of 6.92 µg*hr/ml for a 10 mg/kg dose. Using these data, the F of PMEA from bis-POMPMEA was calculated for each dose level.

9. Pharmacokinetics of bis-POM PMEA in a 6-Month chronic oral toxicity study of bis-POM PMEA in Rats, Lot # 808-GS-93, — January 20, 1998, (94-TOX-0840-003-PK\Project No. 86539)

Groups of male and female rats {strain: Sprague-Dawley Crl:CD(SD)BR; age: 6 weeks} were administered bis-POM PMEA by oral gavage once daily at dose levels of 0 (vehicle control), 0.4 (low), 2.0 (mid) or 10.0 mg/kg/day (high) for 13 or 26 consecutive weeks. Fifteen rats/sex/group were assigned to the main study (26-week) treatment cohort and 10 rats/sex/group were assigned to the interim necropsy (13-week) treatment cohort. Five rats/sex/group were assigned to each control and high dose groups for each post-dose (4-week) non-treatment recovery period. An additional cohort of 14 male rats each in the low, mid and high and 14 female rats (high) were designated for toxicokinetic analysis only. Toxicokinetic blood samples (2 rats/time point) were taken pre-dose and at 1, 2, 4, 6 and 12 hr post-dose on study day 1 and during weeks 12 and 26. The blood samples were processed for plasma and concentrations of PMEA in plasma were determined using a validated — method with fluorescence derivatization. Results: are shown in Table 5. Following repeated oral administration of bis-POM PMEA to rats over the dose range of 0.4 to 10 mg/kg/day, systemic exposure (with respect to both Cmax and AUC) to bis-POM PMEA was not dose proportional at the 2 and 10 mg/kg/day dose levels. The estimated bioavailabilities of PMEA from bis-POM PMEA at the 10 mg/kg/day dose level was 3.5 times lower than at the 2 mg/kg/day dose level.

Table 5
Pharmacokinetic parameters for PMEA in plasma following repeated

daily oral administrations of bis-POM PMEA in rats

Parameters	Dose (mg/kg/day)								
	0.4			2			10		
Week	1	12	26	1	12	26	1	12	26
Cmax (µg/ml)	0.083	0.084	0.136	0.284	0.36	0.45	0.323	0.295	0.503
Tmax (hr)	1	1	2	1	1	1	1	1	1
Half-life (hr)	-	-	-	6.65	2.39	3.49	3.39	5.74	4.89
$AUC_{0-\infty}$ (µg*hr/ml)	-	-	-	1.51	1.43	1.68	1.27	2.1	1.96
MRT (hr)	-	-	-	8.19	3.97	4.95	4.85	8.8	6.27
F (%) using historical AUC_{iv} values	-	-	-	76.1	72	85.7	12.9	21.2	20.3

- = unable to assess the parameter

10. Pharmacokinetics of adefovir in an oral carcinogenicity study of adefovir dipivoxil in the rat (a report of analysis of data from study 96-TOX-0940-003, January 25, 1999, (96-TOX-0840-003-PK)

Plasma levels of adefovir were determined in male and female rats at days 1 and 30 of a 2-year carcinogenicity study involving daily oral adefovir dipivoxil dosing at 0 (vehicle controls), 0.5 (low), 1.5 (mid) or 5 mg/kg/day (high). Blood samples were collected from rats (2 sex/timepoint) by orbital bleeding at 0.25, 0.5, 1, 2, 4, 8 and 24 hr post dosing. Concentrations of adefovir in plasma samples were determined using a validated method. Results: mean pharmacokinetic parameters of adefovir in rat plasma are shown in Table 6. Maximum plasma adefovir levels of all dose groups were achieved between 0.5 and 1 hr after dosing on days 1 and 30. For the 1.5 and 5 mg/kg/day dose levels at day 1, AUC values were apparently dose proportional.

Table 6

Mean pharmacokinetic parameters for adefovir in rat plasma at days 1 and 30 following daily oral adefovir dipivoxil dosing at 0.5, 1.5 and 5 mg/kg/day

Parameters	Dose (mg/kg/day)					
	0.5		1.5		5	
Day	1	30	1	30	1	30

Cmax (µg/ml)	0.02	0.05	0.09	0.11	0.27	0.32
Tmax (hr)	0.5	0.5	1	1	0.5	0.5
$AUC_{(0-t)}$ (µg*hr/ml)	nd	nd	0.21	nd	0.9	nd

nd = not determined due to limited quantifiable data points.

11. Determination of adefovir dipivoxil in hepatic portal blood following oral administration to rats, January 6, 1999, (97-DDM-0840-002)

^{14}C-adefovir dipivoxil (10 mg eq/kg of adefovir: 500 µCi/kg) as a single oral dose was administered via oral gavage to rats (n=4) cannulated in the hepatic portal vein. Blood samples were obtained from the portal vein to determine the intestinal absorption of adefovir dipivoxil into the portal circulation. In a second experiment, three rats received a single oral dose of adefovir dipivoxil (10 mg eq/kg of adefovir: 500 µCi/kg) via oral gavage. Concentrations of adefovir were monitored both in the portal and systemic circulation of these animals. Concentrations of total radioactivity in blood was determined by direct scintillation counting. Quenched blood samples were centrifuged and supernatant was analyzed for metabolites by a validated [redacted] analysis with radioactive flow detection.

Results: adefovir was the only radioactive species detected in portal or systemic blood samples indicating rapid conversion of adefovir dipivoxil to adefovir prior to reaching the systemic circulation. In the first experiment, the mean $AUC_{(0-2\ hr)}$ for adefovir in portal blood samples was 0.48 ± 0.15 µg*hr/ml. The Cmax values were highly variable (0.23 to 0.58 µg/ml; mean = 0.37 ± 0.15 µg/ml). In the second experiment, the concentration vs time curves for adefovir in blood were similar for both systemic and portal venous samples indicating no further hepatic metabolism of adefovir occurred. The adefovir AUC was 0.35 ± 0.07 µg/ml in portal blood and 0.28 ± 0.04 µg/ml in systemic blood. The corresponding Cmax values were 0.24 ± 0.07 µg/ml and 0.18 ± 0.04 µg/ml, respectively.

Comments: These data suggested that adefovir dipivoxil was absorbed following the oral administration and rapidly metabolized to adefovir prior to reaching the portal circulation in the rat. No further hepatic metabolism of adefovir was observed. The primary site of degradation of adefovir dipivoxil following the oral administration in rats appeared to be within the intestinal epithelial cells.

12. Concentration of adefovir in plasma at 2 hours following an oral dose of adefovir dipivoxil in a 14-day oral gavage toxicity

study of bis-POM PMEA in the albino rat, Lot # TX840-97-05, [redacted] December 30, 1998, (97-TOX-0840-006-PK)

Groups of male Sprague Dawley rats (strain: Crl: CD(SD)BR; weights: 183-201 g; 5 rats/group) were administered bis-POMPMEA via oral gavage at dose levels of 0 (vehicle control), 12 (low) or 37 mg/kg/day (high) for 14 consecutive days. Blood samples were obtained at 2 hr after the dosing on the first day of the study. Concentrations of adefovir in plasma were determined using a validated [redacted] method with fluorescence derivatization. Results: concentrations of adefovir at 2 hr after the first dose were 0.25 and 0.54 µg/ml at the 12 and 37 mg/kg/day dose levels, respectively.

13. Pharmacokinetics of PMEA in rats from a developmental toxicity study of PMEA administered intravenously to presumed pregnant rats (A report of analysis of plasma concentration data from toxicity study # 94-TOX-0393-001), 16 December, 1998, (94-TOX-0393-001-PK)

Groups of presumed pregnant rats (strain: Crl:CD BR VAF/Plus; 25 rats/group) were administered PMEA intravenously once daily at dose levels of 0 (vehicle control), 2.5 (low), 10 (mid) or 20 mg/kg/day (high) during gestation days 6 through 15 to evaluate developmental toxicity and teratogenic potential of the test compound. The toxicokinetic arm consisted of 9 rats/sex/group. Blood samples were obtained at 0, 1, 2, 3, 4, 6 and 12 hr post dosing on days 6 and 15 of presumed gestation. Plasma concentrations of PMEA were determined using a validated [redacted] method. Results: are shown in Table 7. $AUC_{(0-\infty)}$ and C_0 were difficult to estimate and compare between dose levels because the concentration data at 1 hr post dose were inconsistent at the 20 mg/kg/day dose level. Because of PMEA concentrations below the detection limit, pharmacokinetic analysis was not possible for the 2.5 mg/kg/day dose level.

Table 7

Pharmacokinetic parameters for PMEA in plasma following once daily intravenous administration to pregnant rats on day 6 through 15 of gestation.

Dose (mg/kg/day)	2.5 (low)		10 (mid)		20 (high)	
Day after gestation	6	15	6	15	6	15
Tmax (hr)	1	1	1	1	1	1
Cl (µg/ml)	-	-	0.716	0.594	1.83	1.89
T½ (hr)	-	-	3.4	1.36	2.81	1.78

AUC (µg*hr/ml)	-	-	0.819	0.66	1.62	1.57
AUC/Dose	-	-	0.08	0.07	0.08	0.08

14. Oral bioavailability of Adefovir (PMEA) from bis-POM PMEA granules in woodchucks (97-DDM-0840-001)

A group of woodchucks (weight ~ 7 kg) was orally administered bis-POM PMEA granules at a dose of 15 mg/kg in three different suspending vehicles (standard woodchuck chow, apple juice and grape juice). Blood samples were obtained over the course of 24 hr and PMEA plasma levels were determined by a validated [redacted] method. Using allometric scaling of plasma clearance of PMEA vs. body weight, the AUC values for intravenous PMEA in woodchuck was estimated to be 17.6 µg-equiv*hr/ml. Results: the pharmacokinetic parameters are summarized in Table 8.

Table 8

Comparison of mean pharmacokinetic parameters for 3 suspending vehicles of bis-POM PMEA granules at a dose of 15 mg/kg in woodchucks

Vehicle	AUC (µg-equiv*hr/ml)	Cmax (µg/ml)	Tmax (hr)	Calculated F (%)
Woodchuck chow (n=2)	1.41	0.36	4	8
Apple juice (n=4)	1.99	0.32	4	11.3
Grape juice (n=2)	3.33	0.33	5	18.9

15. Bioavailability of PMEA from Three Oral Formulations of the Prodrug bis-POMPMEA in Fasted Male Cynomolgus Monkeys, Lot # 477-24-33, Gilead Science, Foster City, CA, May 19, 1993, (DDM-KC-042793).

The oral bioavailability of PMEA was determined for 3 formulations of bis-POMPMEA in four fasted male cynomolgus monkeys. The three oral formulations of bis-POMPMEA (20 mg/kg) examined included a hydroxypropyl-ß-cyclodxtrin complex (HPBCD), a PEG based co-solvent solution and an aqueous suspension. The oral formulations were compared to an iv PMEA (10.9 mg/kg) formulation in a four-way crossover study in the same monkeys, with a 7-day washout period between the formulations. Plasma samples were obtained over the course of 24 hr and were analyzed by a validated [redacted] method. Plasma levels of PMEA declined in an apparent tri-exponential manner following an iv administration. The observed oral bioavailability of HPBCD complex, PEG solution and suspension were 24.7±6.5%, 27.3±12.3% and 22.2±15.6%, respectively.

Comments: The oral bioavailability of the PEG formulation was greatest, but was not significantly different from the other two formulations.

16. An Open Balance Study of IV ^{14}C-PMEA and Oral ^{14}C-bis-POMPMEA Administered to Cynomolgus Monkeys, Lot # JPS-646-9/11, [redacted] December 22, 1993, ([redacted] GSI-R23-93-190/93-DDM-0840-003)

Two groups of monkeys (one monkey/sex/group) were administered a single iv bolus of ^{14}C-PMEA (G1) at a dose level of 10 mg/kg or ^{14}C-bis-POMPMEA at a single oral gavage dose of 18.3 mg/kg (G2). At 24 hr following the iv injection (G1), the greatest percentage of the recovered ^{14}C activity was observed in the urine; the recoveries were 79.15% and 73.04% for the male and female monkeys, respectively. All other organs or tissues collected contained 0.08% or less of the total administered dose. At 24 hr following the gavage administration (G2), in both male and female monkeys, the major portion of the recovered ^{14}C was observed in the urine and feces. In the male monkey, 87.83% of the administered dose was recovered in the urine and 6.27% was present in the feces. In the female monkey, 18.87% of the dose was found in the urine and 35.84% was recovered in the feces. In both animals, the stomach and colon contained the highest ^{14}C activity of any of the internal tissues collected. In the male and female respectively, 0.64% and 0.45% of the dose was recovered from the stomach, and 0.5% and 0.43% of the total dose was present in the colon.

17. Bioavailability of PMEA from a Prototype Oral Suspension Formulation of the Prodrug bis-POMPMEA in Fasted Male Cynomolgus Monkeys, Lot # 840J93-01, Gilead Science, Foster City, CA, January 3, 1994, (DDM-JPS-121593)

The oral bioavailability of a granulated formulation of bis-POMPMEA (10 mg/kg) was studied in five fasted male monkeys. PMEA levels observed following administration of the oral formulation were compared to those observed following an iv administration of PMEA (10 mg/kg) in the same monkeys, with a 7-day washout period between the formulations. Plasma samples were collected over the course of 24 hr and PMEA levels were determined by a validated [redacted] method. The bioavailability of the formulation was 14.3±6.9%. The plasma concentration vs time profiles for the five animals were highly variable, with mean C_{max} and T_{max} values of 0.31±0.31 µg/ml and 9.0±8.8 hr, respectively.

18. Bis-POMPMEA concentrations from a 4-week oral toxicity study with bis-POMPMEA in Cynomolgus monkeys, Lot # JM-614-5, [redacted] ([redacted]/6511-100/93-TOX-0840-002-BA)

Groups of male and female cynomolgus monkeys (weight: 2.0-3.8 kg; age: adult; 4 animals/sex/group) were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control), 8 (low), 25 (mid) or 75 mg/kg (high) for a period of 28 days. Plasma samples were obtained at 0, 0.5, 1, 2, 4, 6 and 12 hr after dosing on days 1 and 30. The samples were analyzed using a validated —— method. Results: are shown in Table 9. No mono-POM PMEA or bis-POM PMEA were detected in the plasma samples.

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Table 9

Pharmacokinetic parameters for PMEA in plasma following repeated daily oral administrations of bis-POM PMEA in monkeys

Parameters	Dose (mg/kg/day)					
	Day 1			Day 30		
	8	25	75	8	25	75
Cmax (µg/ml)	0.47	*	2.48	0.33	*	2.19
Tmax (hr)	1.25	*	1.38	0.88	*	1.88
Half-life (hr)	-	*	2.36	-	*	2.12
$AUC_{0-\infty}$ (µg*hr/ml)	-	*	8.96	-	*	8.88
AUC/Dose (PMEA)	-	*	0.22	-	*	0.22
F (%) using historical AUC_{iv} values	-	*	13.3	-	*	13.3

- = unable to assess the parameter (concentrations below the minimum quantifiable level for PMEA)
* = sample were not collected

19. Pharmacokinetics of PMEA in a 91-day Oral Toxicity Study with bis-POMPMEA in Cynomolgus Monkeys, Lot # JM-614-70-11, —— September 1, 1994, (— /6511-103/94-TOX-0840-001-BA/P0840-00008)

Groups of male and female cynomolgus monkeys (weight: 2.0-3.7 kg) were administered bis-POMPMEA by oral gavage once daily at dose levels of 0 (vehicle control, 6 animals/sex), 1 (low, 4 animals/sex), 5 (mid, 4 animals/sex) or 25 mg/kg (high, 8 animals/sex) for a period of 13 weeks. For drug absorption determinations, blood was collected at 0, 0.5, 1, 2, 4, 6 and 12 hr post dose on days 1, 25, 53 and 84. Results: pharmacokinetic parameters are summarized in Table 10. Exposure to PMEA following multiple oral doses of bis-POMPMEA over the course of the study was dose-proportional. The approximate oral bioavailability of PMEA from bis-POMPMEA was 26.5, 40.0 and 33.3% at 1, 5 and 25 mg/kg/day, respectively.

Table 10

Pharmacokinetic Parameters for PMEA Following Repeated Oral Administration of bis-POMPMEA to Monkeys

Dose (mg/kg/day)	Parameters	Mean Pharmacokinetic Parameters							
		Day 1		Day 25		Day 53		Day 84	
		♂	♀	♂	♀	♂	♀	♂	♀
1	AUC (µg*hr/ml)	0.07	014	0.22	0.2	0.1	0.5	0.0	0.25
	Cmax (µg/ml)	0.04	0.06	0.05	0.08	0.04	0.08	0.0	0.06
	Tmax (hr)	1.13	0.88	1.25	0.88	1.5	2.13	nd	1.13
	$T_{½}$ (hr)	nd	nd	nd	nd	nd	nd	nd	nd
5	AUC (µg*hr/ml)	0.94	0.85	0.89	0.94	0.85	1.09	0.8	1.0
	Cmax (µg/ml)	0.21	0.26	0.21	0.34	0.18	0.29	0.19	0.2
	Tmax (hr)	1.13	1.25	0.38	0.5	0.75	0.88	1.38	2.0
	$T_{½}$ (hr)	9.8	5.15	7.6	4.1	5.6	9.7	7.2	7.4
25	AUC (µg*hr/ml)	4.13	4.6	4.8	4.13	4.82	5.14	5.4	4.58
	Cmax (µg/ml)	1.88	1.57	1.96	1.11	1.35	1.2	1.59	0.86
	Tmax (hr)	0.81	1.13	0.63	1.44	1.08	1.08	0.75	1.67
	$T_{½}$ (hr)	5.3	6.2	6.5	5.6	3.6	5.0	5.1	6.3

Comments: The observed Cmax at 25 mg/kg/day was significantly lower in females than males on day 25 (p=0.002) and 84 (p=0.025). The corresponding Tmax at this dose was significantly greater in females than males on day 25 (p=0.004) and 84 (p=0.003). However, AUC values were not affected by sex at the dose. This suggested that the rate of absorption of bis-POMPMEA was delayed in females on days 25 and 84, while the extent of absorption remained unaffected.

20. Pharmacokinetics of bis-POM PMEA in a 52-Week Oral Toxicity Study with bis-POM PMEA in Cynomolgus Monkeys, Lot # TX840-96-01, January 23, 1998, (T0840-00019/95-TOX-0840-006-PK)

Groups of male and female cynomolgus monkeys (weight: 2.0-2.9 kg; 5 animals/sex/group) were administered bis-POM PMEA by oral gavage once daily at dose levels of 0 (vehicle control), 0.2 (low), 1.0 (mid) or 5 mg/kg/day (high) for a period of 52 weeks. A recovery cohort (2 animals/sex/group) in the low and high dosage groups were retained for 4-non-dosing weeks following the 52 treatment weeks. For drug absorption determination, blood was collected at 0, 0.5, 1, 2, 4, 6, 12 and 24 hr post dosing on day 1 and during weeks 13, 26, 39 and 52. The blood samples were processed for plasma and concentrations of PMEA in plasma were determined using a validated [redacted] method with fluorescence derivatization. Results: are shown in Tables 11-13. Following the oral dose of 5 mg/kg/day, concentrations of PMEA in plasma peaked at Tmax 0.5 to 1 hr. Thereafter, concentrations declined with half-lives of 5.46 for males and 4.68 for females. Repeated administration of bis-POM PMEA at the 5 mg/kg/day over 52 weeks did not lead to significant changes in AUC values or other pharmacokinetic parameters.

Table 11

Pharmacokinetic parameters for PMEA in plasma following repeated daily oral administrations of bis-POM PMEA (5 mg/kg/day) in monkeys

Parameters	5 mg/kg/day									
Week	1		13		26		39		52	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Cmax (µg/ml)	0.177	0.255	0.21	0.133	0.182	0.228	0.132	0.266	0.14	0.183
Tmax (hr)	0.857	0.857	1.25	1.25	0.75	0.5	0.75	1.25	1.57	1.57
Half-life (hr)	5.46	4.68	3.64	7.98	7.27	10.53	6.71	9.29	5.82	5.15

$AUC_{0-\infty}$ (µg*hr/ml)	1.17	1.12	0.943	1.75	1.25	1.31	1.12	2.69	1.37	1.39
MRT (hr)	8.22	6.79	5.44	13.2	9.74	13.9	10.5	13.5	11.2	8.37
F (%) using historical AUC_{iv} values	13.9	13.3	11.2	20.8	14.9	15.6	13.3	31.9	16.2	16.5

- = unable to assess the parameter

Table 12

Cmax and Tmax for PMEA in plasma following once daily repeated oral administration of 0.2 or 1.0 mg/kg/day bis-POM PMEA in male cynomolgus monkeys

Parameters	Dose (mg/kg/day)									
	0.2					1.0				
Week	1	13	26	39	52	1	13	26	39	52
Cmax (µg/ml)	nd	0.017	nd	nd	0.036	0.053	0.063	0.035	0.16	0.042
Tmax (hr)	nd	0.05	nd	nd	6.0	1	0.5	0.5	0.5	2

nd = not detected

Table 13

Cmax and Tmax for PMEA in plasma following once daily repeated oral administration of 0.2 or 1.0 mg/kg/day bis-POM PMEA in female cynomolgus monkeys

Parameters	Dose (mg/kg/day)									
	0.2					1.0				
Week	1	13	26	39	52	1	13	26	39	52
Cmax (µg/ml)	0.035	0.016	0.035	nd	0.021	0.045	0.047	0.03	0.03	0.039
Tmax (hr)	12	0.5	12	nd	2	0.5	1	0.5	6	0.5

nd = not detected

21. Protein Binding of Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum, Cidofovir Lot # 1966-C-9P), Gilead Sciences, Inc., Foster City, CA, June 9, 1995 (PO504-00039/95-DDM-XXXX-001)

The protein binding of cidofovir, cyclic HPMPC, 9-(2-phosphonylmethoxyethyl) adenine [PMEA] and 9-(2-phosphonylmethoxyproyl) adenine [PMPA] was determined in human plasma and serum using a centrifugation and ultrafiltration method. Five concentrations of each compound were prepared in phosphate buffered saline (PBS), human plasma and serum over the range of [redacted] for cidofovir and cyclic HPMPC, [redacted] for PMEA and [redacted] for PMPA. Results: are shown in Table 14. All the four compounds showed a very low protein binding (< 6%) in either human plasma or serum.

Table 14
Percent Unbound (SD) Cidofovir, Cyclic HPMPC, PMEA and PMPA in Human Plasma and Serum

Compound	Mean Percent Unbound (SD)		
	Human Plasma	Human Serum	PBS
Cidofovir	94.8 (3.8)	99.9 (3.3)	100.6 (1)
Cyclic HPMPC	96.7 (3.9)	95.7 (5.2)	99.3 (4.2)
PMEA	98.2 (6.3)	100.8 (7.2)	100 (1.3)
PMPA	99.3 (3.3)	92.8 (3.6)	99.8 (2.3)

22. A Pilot Study of Bioavailability in the Rat, [redacted] 40085 (PMEA), Lot # 26870-059 and 26614-069a, [redacted] April 12, 1989, ([redacted]-25365)

Two groups of male rats (strain: COBS CD (SD)BR; weight: 150 - 200 g; 15/group; fasted overnight) were given oral (with a 5 ml syringe fitted with 3 inch 20 gauge intubation needle) or iv (2 ml/kg bolus injection into the tail vein with a 1 ml syringe fitted with 3/4 inch 25 gauge needle) dose of PMEA at 30 mg/kg. After the dosing, blood samples were taken from 3 rats (each rat was bled twice; the bleed time was such that no rat was bled less than 15 min after the first bleed) at 5, 10, 20, 40, 60, 90, 120, 180 and 360 min by orbital sinus bleeding. The plasma samples were analyzed by a validated [redacted] method and various pharmacokinetic parameters were estimated. Mean plasma concentrations of PMEA following oral and iv administration are

shown in Table 15.

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Table 15

Rat Plasma Concentrations (Mean ± S.D.) of PMEA (µg/ml) Following a Single Oral and Intravenous Administration of 30 mg/kg of PMEA

Route	Time (min)									
	5	10	20	40	60	90	120	180	240	360
	Concentrations (Mean ± S.D.) of PMEA (µg/ml)									
PO	0.96 ± 0.76	0.45 ± 0.29	0.3 ± 0.05	0.32 ± 0.02	0.32 ± 0.05	0.44 ± 0.12	0.34 ± 0.14	0.31 ± 0.04	0.22 ± 0.05	0.1 ± 0.0
IV	21.5 ± 7.0	31.3 ± 4.8	15.7 ± 4.3	5.9 ± 1.1	5.0 ± 2.5	2.2 ± 0.2	0.64 ± 0.23	0.41 ± 0.03	0.25 ± 0.01	0.2 ± 0.0

As shown Table 15, maximum plasma concentration of about 1 µg/ml was achieved after 5 min of oral dosing. Plasma concentrations decreased biphasically with a terminal half-life after iv dosing estimated to be 156 min, which was about the same determined after oral dosing (149 min). The pharmacokinetic parameters for PMEA following oral and iv dosing are shown in Table 16. The absolute bioavailability of PMEA was 11%. The plasma (iv administration) clearance and volume of distribution were found to be 27 ml/min/kg and 6.2 l/kg, respectively.

Comments: The volume of distribution is about ten times greater than the total body water (0.6 l/kg), and 100 times greater than the mean blood volume ((0.06 l/kg) of this strain of rat, which suggests that there may be extensive localization of PMEA in the tissues.

Table 16

Summary of Rat Pharmacokinetics Parameters for PMEA after a Single Oral and Intravenous Administration of 30 mg/kg of PMEA

Route & Dose	C_{max} (µg/ml)	T_{max} (min)	$T_{1/2}$ (min)	CL (ml/min/kg)	V_{dss} (l/kg)	AUC (µg.min/ml)	F (%)